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# BUB1 serves as a biomarker for poor prognosis in liver hepatocellular carcinoma

Lili Zhang[1,2*], Yuzheng Zhuge[1*] and Jingbin Ni[2]

## Abstract

**Background** Hepatocellular carcinoma (HCC) is the most frequent kind of liver cancer with high morbidity and mortality rates worldwide. Altered expression of BUB1 (budding uninhibited by benzimidazole 1) gene leads to chromosome instability and aneuploidy. This study investigated the expression of BUB1 and its prognostic value as well as its correlation with immune cell infiltration and immune checkpoints in HCC.

**Results** Using the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases, we found that BUB1 was up-regulated in HCC, thus prompting us to validate this observation by immunohistochemistry on 57 HCC paraffin embedded tissues from Wuxi No.2 People's Hospital. Kaplan-Meier survival analysis revealed that HCC patients with high BUB1 expression had shorter overall survival (OS) time as well as progression-free interval (PFI), and disease-specific survival (DSS) time compared to the patients with low BUB1 expression. Besides, STRING database showed that the top 10 co-expression genes were mainly involved in the regulation of cell division during the mitosis. Gene Ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that BUB1 had a connection to cancer related pathways. Lastly, The Tumor Immune Estimation Resource (TIMER) analysis found that BUB1 was positively related to immune cell infiltration and some immune checkpoint gene in HCC.

**Clinical trial number** Not applicable.

**Conclusions** Our present study demonstrated that BUB1 is a potential prognostic biomarker, and BUB1 may play a role in the tumor immune microenvironment in HCC.

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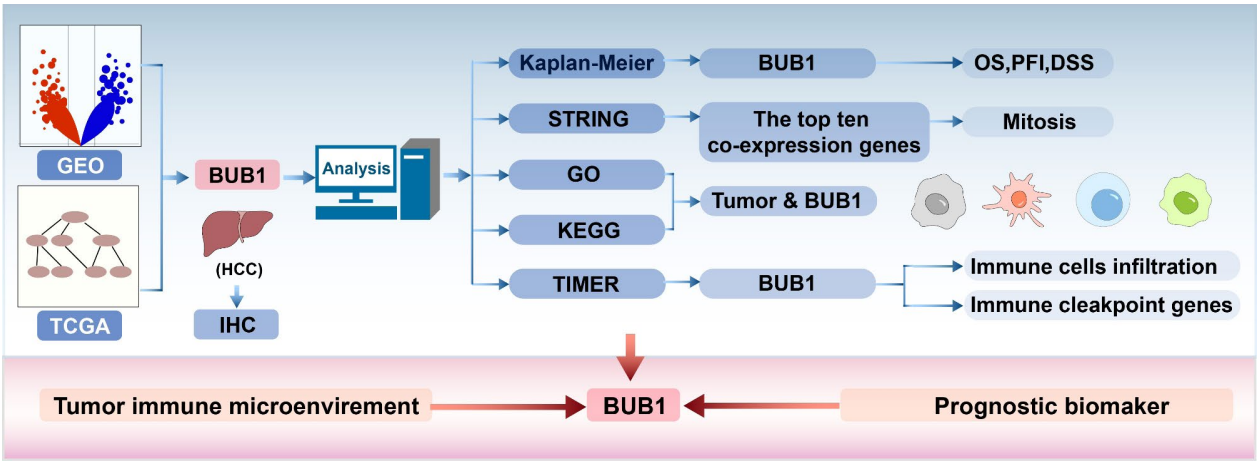
**Graphical Abstract**




**Keywords** BUB1 gene, Bioinformatics, Liver hepatocellular carcinoma, Survival, Immune regulation

## Background

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and the Third leading cause of cancer mortality worldwide [1]. Despite recent advances in HCC early diagnosis and specialized HCC treatments, the long-term survival of HCC patients is still poor. Only 15% of patients are eligible for potentially curative treatments such as surgical resection or transplantation since most patients have been diagnosed in the advanced stage [2]. However, up to 80% patients experience recurrence even after curative resection [3]. Dysregulation of genes could lead to the progression and recurrence of the tumor [4]. It is imperative to identify the molecular mechanisms and regulatory network of HCC to discover therapeutic targets to block the occurrence and development of the disease.

Moreover, establishing a model for predicting HCC prognosis is similarly significant. Recently, the revolutionary advent of the role of immune checkpoint inhibitors(ICI)for advanced HCC has been recognized. ICI immunotherapy targeting either the programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) or cytotoxic T-lymphocyte (CTLA-4) pathways has achieved unprecedented results for many types of cancers [5].

BUB1 (budding uninhibited by benzimidazole 1) is a conserved mitotic checkpoint serine/threonine kinase that plays a central role in spindle checkpoint signaling and regulation of chromosome alignment [6]. Recently, studies have reported BUB1 as an oncogene or tumor suppressor gene in cancers, such as pancreatic ductal adenocarcinoma [7], gastric adenocarcinomas [8], and breast cancer [9].

In this study, we elucidated the value of BUB1 in predicting the prognosis of HCC and its correlation with immune checkpoints by bioinformatics analysis from public databases.

## Methods

### Data

RNA-sequencing expression profiles and corresponding clinical data were acquired from the TCGA database( https://www.cancer.gov/ccg/research/genome-sequencing/tcga ). Gene expression profiles of GSE84402 [10] and GSE101685 [11] were obtained from the NCBI GEO ( http://www.ncbi.nlm.nih.gov/geo ) database, which included 38 tumor samples and 22 normal samples from 60 different patients.

### Analysis of differential expression and clinical values

The expression of BUB1 in pan-cancer and HCC was analyzed. We assessed diagnostic and survival values of BUB1 expression through the area under the curve and survival analysis, histological grade, T stage, pathologic stage, AFP (alpha-fetoprotein) content, and tumor status. Besides, overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) were used as prognostic factors.

### Biological functional analysis

A protein-protein interaction (PPI) network of BUB1 co-expressed genes was identified through the STRING database (https://cn.string-db.org/), and high confidence was set as 0.7. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed on co-expressed genes with R clusterProfiler

to explore possible biological functions and signaling pathways affected by BUB1. GO analysis included biological process, cell composition, and molecular function, with $P<0.05$ as statistically significant. TCGA data were analyzed with gene-set enrichment analysis. We further analyzed cancer-related pathways of BUB1 and its co-expressed genes through gene set variation analysis (GSVA) via GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/).

#### Immunohistochemistry

To validate the reliability of the predictions from the public databases, we collected 57 cases of resected HCC from January 2021 to December 2022 at WuXi No.2 People's Hospital. No preoperative treatment was administered to any of the patients. Retrospectively, clinical pathological data as well as follow-up survival statistics were collected. This study was in accordance with the Declaration of Helsinki was approved by the hospital' ethics committee(2021-Y-2).

Immunohistochemistry analysis was performed on tissue microarray by using antibody against BUB1 (Abcam, U.S.A, ab 195268)(1:100), and treated with a secondary anti-body (Abcam, U.S.A, ab 97051)(1:300).Two experienced pathologists independently assessed all cases and the histological diagnosis was confirmed in full agreement.

We use Immunoreactive Score Method.The staining intensity was scored as 0 (negative, blue), 1 (weak, light brown), 2 (moderate, brown) or 3 (strong, dark brown). The staining proportion was scored as 0 (< 5%), 1 (6–25%), 2 (26–50%), 3 (51–75%) or 4 (>75%). The intensity and proportion scores were multiplied to calculate the final immunoreactivity score. For statistical analysis, groups were defined as the highly expressed group (total score ≥ 4,++~+++) and low expressed group (total score < 4,-~+).

#### Correlation analysis of BUB1 with immune cell infiltration and immune checkpoints

Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) is a public website for a comprehensive analysis of immune information of various tumor types. It encompassed 10,897 samples across 32 cancer types from the TCGA database to assess the abundance of tumor-infiltrated immune cells (CD8 + T cells, CD4 + T cells, B cells, and macrophages, neutrophils, and dendritic cells). The correlation of BUB1 expression with immune checkpoints in HCC was evaluated using TIMER and R with the ggplot2 package based on TCGA.

#### Statistical analysis

R 3.6.3 software was used for statistical analysis with the packages ggplot2, pROC, survival, survminer, clusterProfiler, and RMS. The chi-square test was used to assess differences between categorical variables and paired or unpaired t-tests used for continuous variables. The Wilcoxon rank-sum test was used if Shapiro–Wilk normality testing yielded significance. Survival was analyzed using Kaplan–Meier curves and log-rank tests. All tests were two-sided. $P<0.05$ was considered statistically significant.

## Results

#### Correlation between BUB1 expression and clinical characteristics in HCC

We compared the mRNA expression levels of BUB1 between pan-cancers and corresponding normal tissues based on RNA-sequence data from the TCGA database. It showed that BUB1 levels were higher in 20 cancer samples compared with normal samples (Fig. 1A). We downloaded the data of 374 HCC patients, unpaired expression data analyses showed that BUB1 expression levels in HCC tissues were higher than those in normal tissues (Fig. 1B). Then the expression of BUB1 was divided into the high and low expression groups.Chi-square tests were used to analyze clinical variables between the two groups. Based on the gene profile and clinical data extracted from TCGA, the distribution of BUB1 showed a significant difference among the tumor grade and stages, OS event, AFP content and tumor status. BUB1 was highly expressed in tumor grades classified as G3&G4 compared to G1&G2 (Fig. 1C) as well as in tumor stages T3&T4 compared to T1&T2 (Fig. 1D) and pathological stages III&IV compared to stages I&II (Fig. 1E). Similarly, BUB1 was increased in dead patients (Fig. 1F). Moreover, BUB1 expression increased in patients with higher AFP content (Fig. 1G) or patients with tumor (Fig. 1H).

For our own data (Table 1), immunohistochemical score < = 4 was a low expression of BUB1, whereas >4 was a high expression of BUB1. We observed that BUB1 expression was positively correlated with the ki67 status ( $P<0.05$), which indicate the invasive ability of tumors, and evaluate the prognosis of cancer. However, BUB1 expression exhibited no significant relevance with gender, age, tumor size, stage status, which may be caused by an insufficient number of cases (all $P>0.05$).

#### Validation of elevated BUB1 expression in HCC tissues

In order to validate the predicted overexpression of BUB1 in HCC tissues, two mRNA microarray datasets (GSE84402 and GSE101685) were downloaded from the GEO database. The expression profiles of BUB1 were analyzed. The results confirmed that BUB1 expression was indeed upregulated in HCC tissues compared to adjacent normal liver tissue in GSE84402 and GSE101685 (Fig. 2A and B). Besides, we also performed immunohistochemical staining and the results indicated a higher positivity

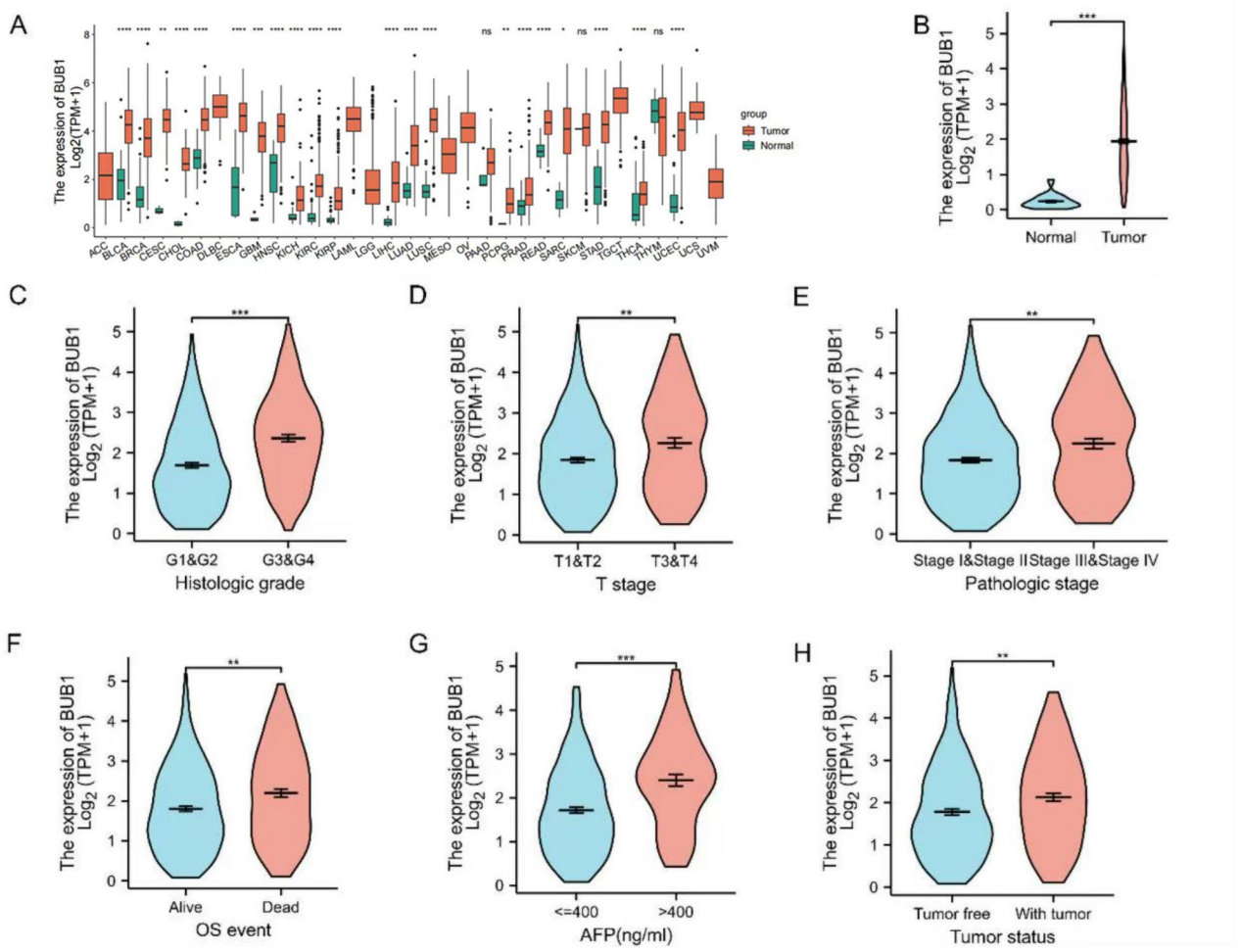



**Fig. 1** BUB1 expression status in various cancers, especially in HCC (LIHC,Liver hepatocellular carcinoma) (A) The expression level of BUB1 in different types of tumor tissues and normal tissues in TCGA database; (B) BUB1 mRNA expression comparison between normal and tumor tissues in the HCC; (C-H) RRM2 expression distribution analyses stratified based onhistologic grade, pathologic stage, T classification, OS event, AFP content and tumor status (Kruskal-Wallis test). (n=347,**, P < 0.01; ***, P < 0.001)

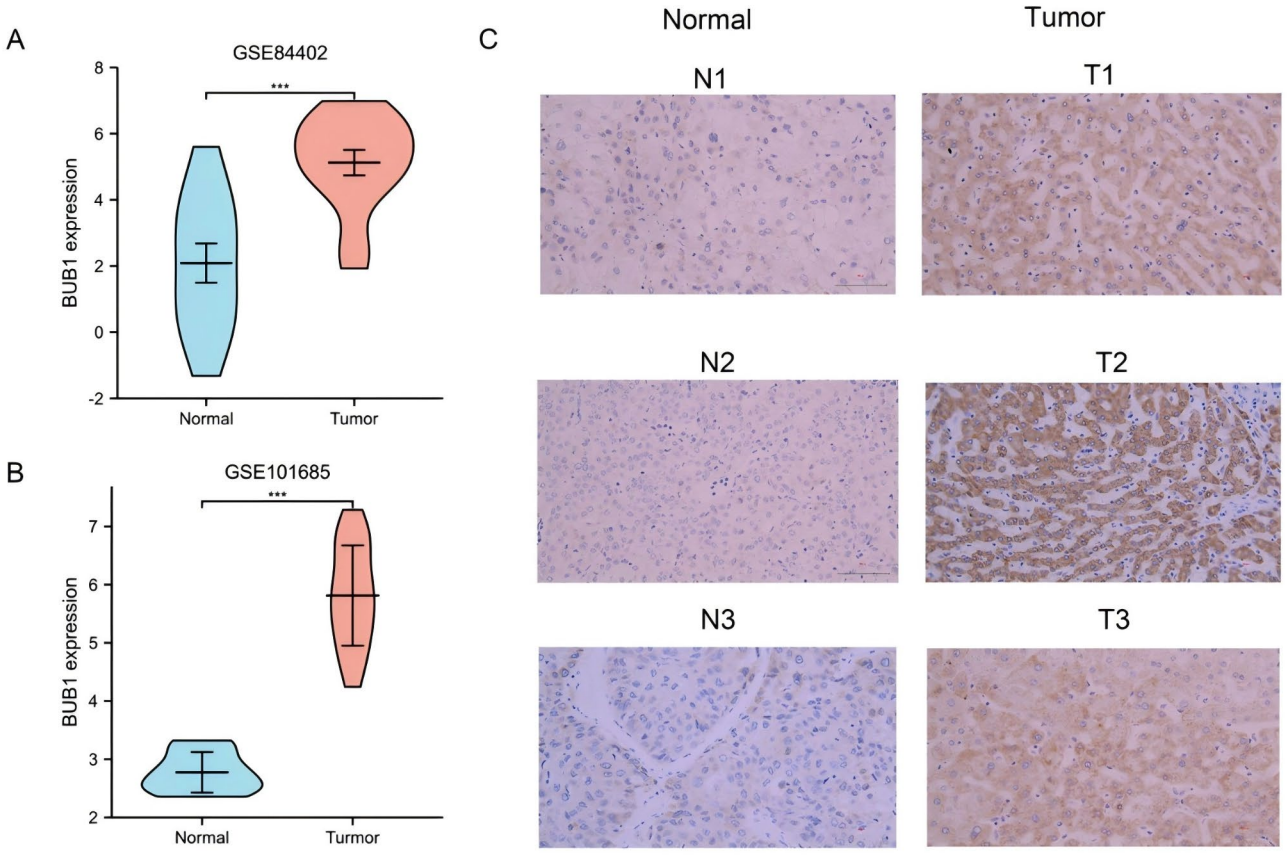
**Table 1** The relation between BUB1 and clinical characteristics of the HCC patients

| Characteristic | BUB1 expression | | *r*(Spearman) | *P* value |
|---|---|---|---|---|
| | <=4 | >4 | | |
| n | 43 | 14 | | |
| Age | | | 0.211 | |
| <=60 | 14 | 3 | | |
| >60 | 29 | 11 | | |
| Gender | | | -0.009 | |
| Male | 11 | 3 | | |
| Female | 32 | 11 | | |
| Dimer | | | -0.064 | |
| <=5 | 19 | 10 | | |
| >5 | 24 | 4 | | |
| Ki67 | | | 0.511 | < 0.01 |
| <=50% | 26 | 3 | | |
| >50% | 17 | 11 | | |
| Stage | | | 0.155 | |
| Stage 1 and 2 | 32 | 11 | | |
| Stage 3 and 4 | 11 | 3 | | |

of BUB1 expression in terms of density and intensity in tumor tissues than in adjacent noncancerous tissues (Fig. 2C). Further analysis of BUB1 protein expression found that BUB1 was expressed in both, with a positive rate of 100% in tumors and 64.9% in adjacent tissues. However, the staining intensity was higher in tumors, with intensely positive rates of 61.4% in tumors versus 19.3% in adjacent tissues (Fig. 2D; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

### Higher BUB1 mRNA expression indicates a worse survival rate

The correlation between BUB1 expression and prognosis in HCC was analyzed using Kaplan-Meier method. In the TCGA database, survival curves showed that elevated BUB1 mRNA levels in HCC patients significantly correlated with poorer OS (HR 1.75, 95% CI 1.24–2.49), PFI (HR 1.7, 95% CI 1.27–2.28) and DSS (HR 2.31, 95% CI 1.46–3.68) (Fig. 3A-C). The area under the




D

| Tissue | No. of cases | BUB1 expression | | | | Positive rate | Intensely positive rate |
|---|---|---|---|---|---|---|---|
| | | - | + | ++ | +++ | | |
| Paracancerous | 57 | 20 | 26 | 8 | 3 | 64.9% | 19.3% |
| Cancer | 57 | 0 | 22 | 21 | 14 | 100%* | 61.4%** |

**Fig. 2** The expression of BUB1 in tumor and paracancer. (A-B) the expression of BUB1 in tumor and adjacent normal tissue in GEO datasets. (C) Representative immunohistochemical staining of BUB1 in HCC and adjacent normal tissue. (D) Positive rate of immunohistochemical staining of BUB1 in HCC and adjacent normal tissue. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

receiver-operating characteristic curve of BUB1 expression in HCC was 0.969 (95% CI 0.953–0.985, Fig. 3D).

#### Construction of protein interaction networks

The functional protein interactions are essential for the molecular and metabolic mechanisms in malignancy. The genes most related to BUB1 can be found by constructing PPI network with STRING database and visualized and analyzing with Cytoscape software.The top five positively BUB1 related genes were TPX2, CKAP2L, DLGAP5, BUB1B and TOP2A, while negatively BUB1 related genes were SLC27A5, LDHD, HP, RNASE4 and HPD (Fig. 4A). Therefore, we searched for proteins that interact with the BUB1 STRING tool and constructed the PPI network. These co-expression genes included AURKB, BUB3, NDC80, CCNB2, BUB1B, MAD2L1, CENPE, PLK1, NUF2, CASC5 (Fig. 4B). These 10 genes are mainly involved in the regulation of cell division during the mitosis [12]. Nearly all of them were confirmed to be highly expressed in tumors. GO and KEGG showed that BUB1 was mainly involved in the chromosome separation, nuclear division, organelle fission, condensed

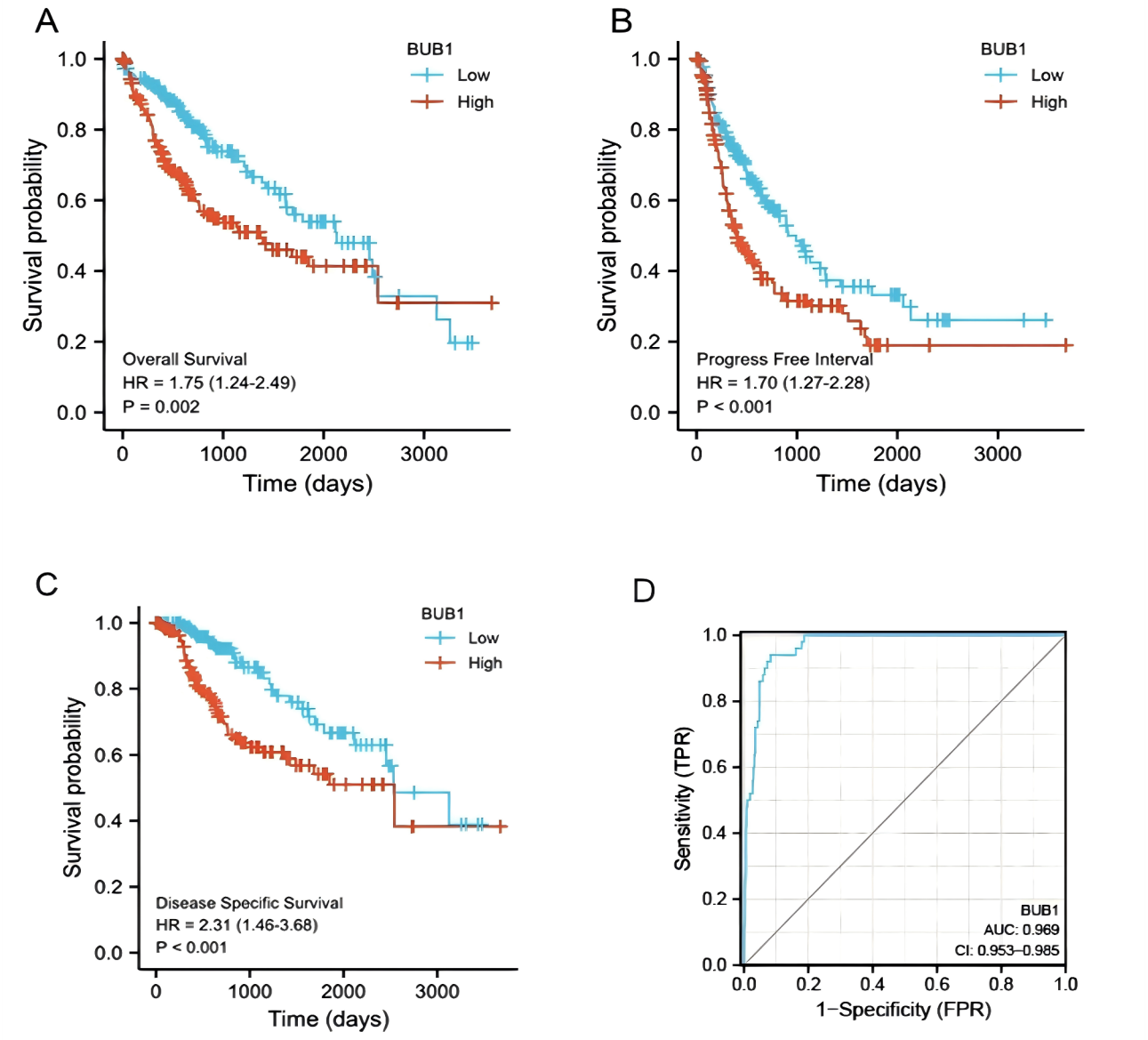



**Fig. 3** Survival analysis in HCC patients with high and low BUB1 expression level. (**A**) relationship between BUB1 expression and overall survival in HCC; (**B**) relationship between BUB1 expression and progression-free interval in HCC; (**C**) relationship between BUB1 expression and disease-specific survival; (**D**) receiver-operating characteristic curve of BUB1 expression in HCC

chromosome outer kinetochore, spindle, condensed nuclear chromosome kinetochore, cell cycle, ubiquinone and other terpenoid-quinone biosynthesis and primary bile acid biosynthesis (Fig. 4C). On the other hand, the significantly enriched cancer-related pathways were the apoptosis, cell cycle, DNA damage and EMT (Fig. 4D).

### Correlation analysis between BUB1 expression and immune infiltration in HCC

It is known that hepatitis or alcoholism can cause inflammation, which in turn leads to HCC tumorigenesis [13], However, it remains unknown what role the immune system plays in the progression of cancer and the prognosis. It has been reported that the extent of the immune cell infiltration into the tumor is highly relevant to the prognosis in HCC and other malignancies [14, 15]. Therefore, we explored the association between BUB1 expression and infiltrating immune cells in HCC using the TIMER database. Our results showed that BUB1 expression was positively correlated with the levels of different infiltrating immune cell types, including B cells ($r = 0.477$, $P = 6.07e-21$), macrophages ($r = 0.462$, $P = 1.91e-19$), neutrophils ($r = 0.424$, $P = 1.71e-16$) and dendritic cells ($r = 0.478$, $P = 7.74e-21$) (Fig. 5A).

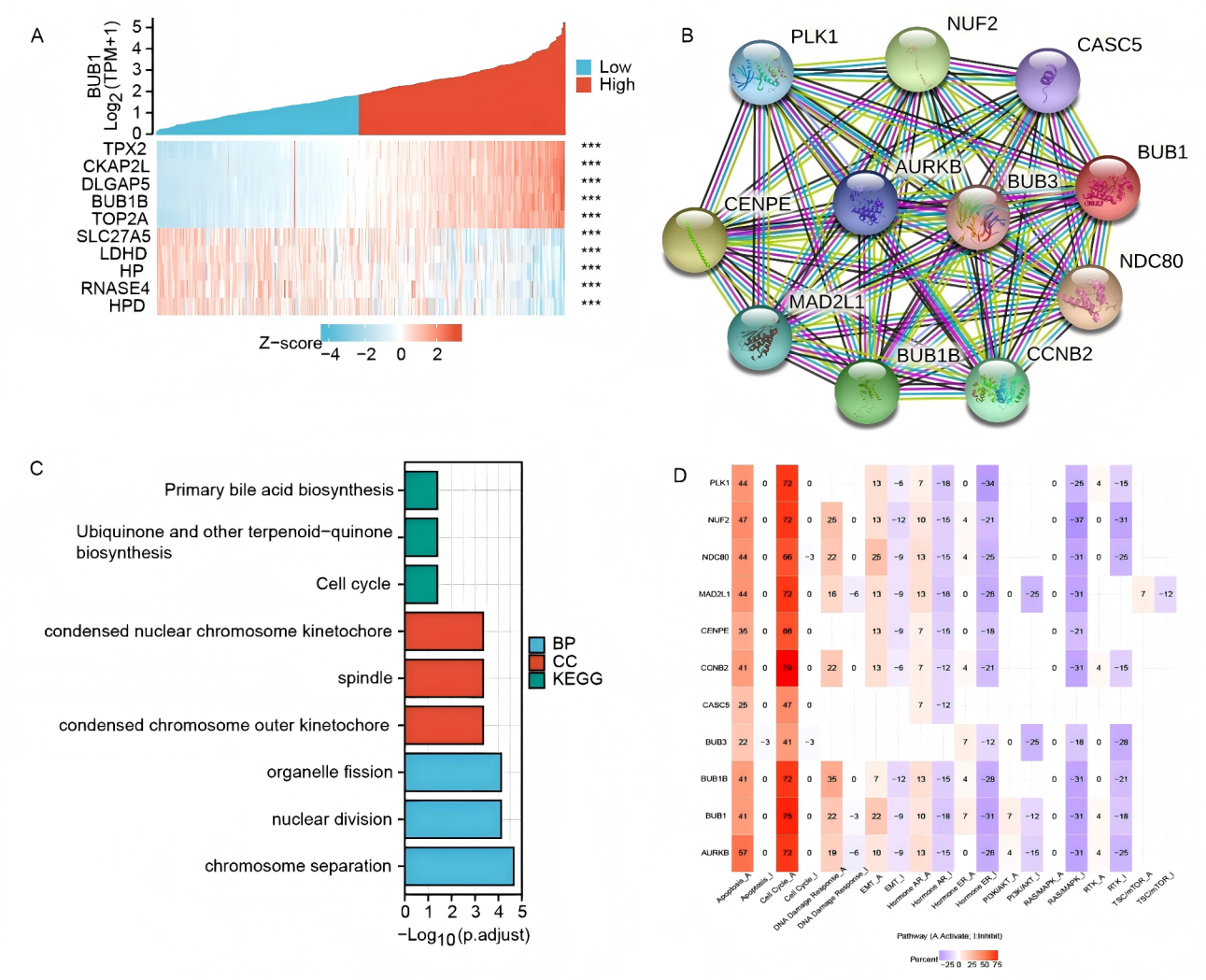



**Fig. 4** Functional enrichment analysis and the top ten genes through protein–protein interaction network. (**A**) Heat map of BUB1 and its top ten positively or negatively correlated genes; (**B**) co-expression genes through protein–protein interaction network; (**C**) GO and KEGG analysis; (**D**) association between GSVA score and activity of cancer-related pathways in HCC

### Relationship between BUB1 expression and immune checkpoints in HCC

Immune checkpoints are responsible for tumor immune escape. Considering the potential oncogenic role of BUB1 in HCC, the relationship between BUB1 expression and 46 immune checkpoint gene [16] was analyzed. The results are shown in Fig. 5B, and the 10 immune checkpoint genes most related to BUB1 expression were CD276 (cor: 0.563), TNFSF4 (cor: 0.498), CD80 (cor: 0.436), HHLA2 (cor: 0.392), TNFSF15 (cor: 0.392), TNFRSF4 (cor: 0.356), CTLA4 (cor: 0.352), HAVCR2 (cor: 0.352), LAIR1 (cor: 0.345), TNFRSF18 (cor: 0.344).

## Discussion

BUB1 is well-known as a key component of the mitotic checkpoint. Mis-regulation of this spindle assembly checkpoint by mutation or aberrant expression is associated with aneuploidy and a wide spectrum of human cancers. BUB1 mutations, including deletions and point mutations, as well as the abnormal BUB1 gene expression are found in cancer tissues and cancer cell lines [17, 18]. Previous studies have shown that BUB1 was significantlyoverexpressed in various human malignancies [8, 9]. In our study, we combined public databases and 57 paired HCC and para-carcinoma tissues from our center and

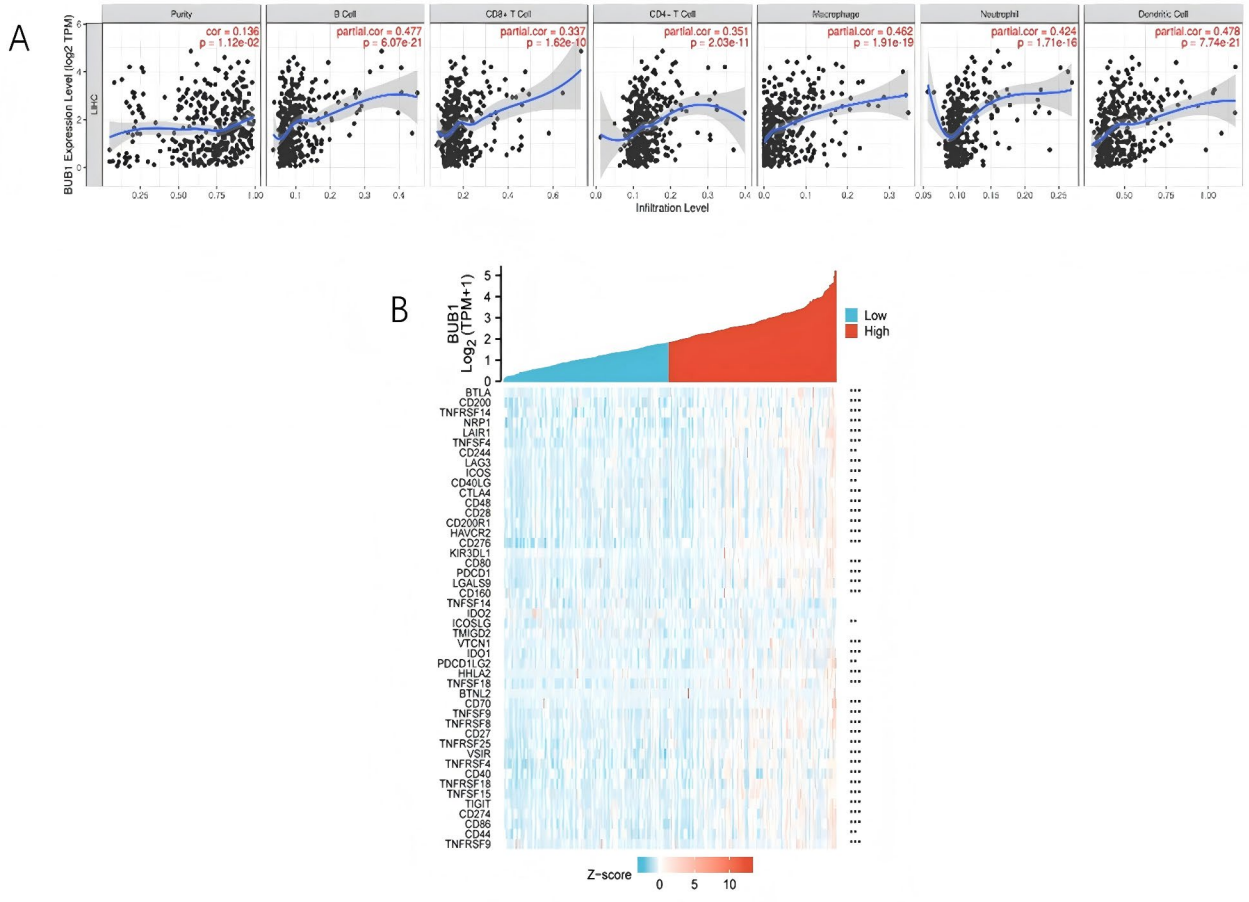



**Fig. 5** BUB1 expression was associated with purity, immune cell infiltration and several immune checkpoint genes in HCC. (**A**) The scatter plots illustrate the relationship between BUB1 and immune cell infiltration, The positively related cells include B cells, macrophages, neutrophils and dendritic cells. (**B**). The heatmap displays the values of chekpoint genes related to BUB1 expression.The colors range from blue to red, typically representing a transition from lower to higher values.The 10 top high-value genes are CD276, TNFSF4, CD80, HHLA2, TNFSF15, TNFRSF4, CTLA4 HAVCR2, LAIR1 TNFRSF18

found that BUB1 expression levels in hepatocellular carcinoma were both higher than that in the normal liver, which first defined the expression pattern of BUB1 in HCC. Then, from the TCGA database, BUB1 high expression was not only correlated with adverse clinicopathological features, including advanced T stage, pathologic stage, tumor status, histologic grade, high AFP content, but also poorer survival of HCC patients, thus suggesting that BUB1 may exhibit a cancer-promoting role in HCC. However, from our own data, all we found was BUB1 expression positively correlated with ki67 status. The inconsistency is mainly due to the shortage of sample size, which needs to be increased in further research.

To investigate the signaling pathways correlated with BUB1 expression, proteins that interact with BUB1 molecule in HCC were analyzed by PPI, and functional analysis of BUB1 was performed by GO and KEGG. The co-expression genes included AURKB, BUB3, NDC80, CCNB2, BUB1B, MAD2L1, CENPE, PLK1, NUF2, CASC5. As expected, the co-expressed genes were primarily related to cell cycle functions and pathways and almost all of them are usually detected in human cancers. Some genes reported to exert oncogenic effects in HCC. Studies have reported the overexpression of AURKB (Aurora kinase B) in HCC tissues and cell lines, which was correlated with poor clinicopathologic characteristics [19]. The expression of NDC80 is also upregulated in HCC tissues, knockdown of NDC80 suppresses HBV replication [20]. BUB1B was significantly higher in HCC cell lines and related to the malignant behavior of tumor cells [21, 22].

GO and KEGG pathway analyzes revealed BUB1 is mainly related to processes and pathways associated with the maintenance of chromosomal stability (cell cycle and division), thereby participating in genomic damage (uncontrolled cell proliferation). Besides, BUB1 is also significantly enriched in EMT(epithelial mesenchymal transition). EMT is believed to play a vital role in invasion, metastasis, chemo-resistance and processes that are

involved in cancer cell aggressiveness [23]. These findings validated the role of BUB1 in HCC initiation and evolution. However, in vivo studies are needed to confirm the correlation between these processes and pathways in regulating BUB1 function in HCC.

HCC is a typical inflammation-related cancer, immune evasion is one of the features occurring during the occurrence and progression of the malignance [24]. Besides, HCC is a chemotherapy-resistant disease. Many HCC patients are diagnosed at relatively advanced stages, so conventional treatments are often less than ideal. Immunotherapy is one such therapy that functions differently from conventional treatments and show positive responses in some patients recently [25]. Our study suggested a significantly positive relationship between the expression levels of BUB1 and infiltration levels of B cells, macrophages, neutrophils and dendritic cells.The results indicated that modulating BUB1 expression could potentially alter the composition of immune cells in the tumor microenvironment, thereby enhancing the efficacy of immunotherapy. Immune checkpoint-targeting agents have been screened and clinically applied to treat cancers. Targeted blockade of PD-1 or CTLA-4 can activate T cells to destroy tumors [26–28]. Clinical trials of immune checkpoint inhibitors in HCC, such as tremelimumab (anti-CTLA4), nivolumab (anti-PD1), and durvalumab (anti-PDL1), have seen survival benefits [29, 30]. In our study, we found a positive correlation between BUB1 and several immune checkpoint genes. CD276 and HHLA2, like PD1, belong to the B7 family, TNFSF4, TNFSF15, TNFRSF4,TNFRSF18, are known as the tumor necrosis factor (receptor) superfamily members, and CD80, they were described as co-stimulatory molecules for T cell activation. These correlations suggest that BUB1 may influence the tumor microenvironment by modulating the expression of immune checkpoints, which are critical targets in immunotherapy.Current immunotherapy strategies for HCC primarily focus on immune checkpoint inhibitors, such as anti-PD-1/PD-L1 and anti-CTLA-4 antibodies [29, 30]. These therapies aim to enhance the immune system's ability to recognize and attack cancer cells by blocking inhibitory signals. The association of BUB1 with immune checkpoints implies that targeting BUB1 could potentially enhance the efficacy of existing immunotherapies by modulating the tumor immune microenvironment.Further research is needed to explore the mechanisms by which BUB1 influences immune checkpoint expression and immune cell infiltration in HCC.

## Conclusions

We identified BUB1 as a potential biomarker with prognostic significance in patients with HCC. Besides, we found an association between BUB1 expression and immune cell infiltration and checkpoint genes. These findings provided further insights into the pathogenesis of HCC and the role of BUB1 in immunotherapy for HCC. Further studies are worthwhile to explore the exact functions of BUB1 in HCC cells to investigate their potential as therapeutic targets.

### Abbreviations

| | |
|---|---|
| HCC | Liver hepatocellular carcinoma GEO gene expression omnibus |
| TCGA | The Cancer Genome Atlas OS overall survival |
| PFI | Progression-free interval DSS disease-specific survival |
| GO | Gene Ontology KEGG Kyoto Encyclopedia of Genes and Genomes |
| TIMER | Tumor Immune Estimation Resource ICI immune checkpoint inhibitor |
| PPI | Protein-protein interaction |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-025-00698-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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### Author contributions

Lili Zhang performed the research, analysed the results, wrote manuscript and made revision of the manuscript.Yuzheng Zhuge designed the research study. Jing bin Ni analysed the results.

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### Data availability

No datasets were generated or analysed during the current study.

## Declarations

### Ethics approval and consent to participate

This study was approved by the ethics committees of WuXi No.2 People's Hospital (2021-Y-2) and received a waiver for informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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